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**13. ABSTRACT** ***(Maximum 200 Words)***

Our recent findings suggest that keratinocyte growth factor (KGF), the seventh member of the fibroblast growth factor family, is capable of acting as a mediator of the stimulatory effects of estrogen in human breast cancer cells. Interestingly, estrogen stimulates KGF expression. This role of estrogens in the regulation of KGF expression is intriguing because hormonal stimulation is an essential factor in the carcinogenesis of human breast cells, especially during early stages. Therefore, it is possible that KGF could be intimately involved in both physiological and pathological processes in human breast tissue. Thus, the ability to interrupt the stimulatory effects of estrogen in human breast cancer cells at the level of KGF holds potential value as a strategy for development of as a potential therapeutic agent for breast cancer. Consequently, we have proposed to synthesize potential KGF antagonists, which will then be evaluated for efficacy *in vitro* assay systems. During the first year of this funding period, we have concentrated in peptide antagonist synthesis and development of an assay system. There were unexpected difficulties on the peptide syntheses, however, we have hound a way to syntheses those peptides.

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature 10/10/00 Date

## Table of Contents

## Development of KGF antagonist as a breast cancer therapeutic agent

## INTRODUCTION

This study focuses to develop an effective peptide-antagonist as a KGF-receptor for breast cancer therapeutic.

### *Task 1: Synthesise of nine hexacosapeptides as KGF antagonists (Months 1-2)*

*Task 1.1: Analyses of synthetic nine hexacosapeptides will be performed (Month 2)*

The syntheses of hexacosapeptides were extremely difficult mostly due to those amino acid sequences. After several attempts, we have modified the synthesis approach. We have successfully synthesized 11 peptides, which still span entire of human KGF protein. The list of synthetic peptides and those purities and yields of Peptides are shown below.

```
hKGF-1:   ACNDMTPEQMATNVNCSSPE (a.a.: 31-50)
hKGF-2:   NCSSPERHTRSYDYMEGGDI (a.a.: 45-64)
hKGF-3:   EGGDIRVRRLFCRTQWYLRI (a.a.: 59-78)
hKGF-4:   TQWYLRIDKRGKVKGTQEMK (a.a.: 73-92)
hKGF-5:   TQEMKNNYNIMEIRTVAVGI (a.a.: 87-106)
hKGF-6:   RTVAVGIVAIKGVESEFYLA (a.a.: 101-120)
hKGF-7:   SEFYLAMNKEGKLYAKKECN (a.a.: 115-134)
hKGF-8:   AKKECNEDCNFKELILENHYNT (a.a.: 129-150)
hKGF-9:   ILENHYNTYASAKWTHNGGE (a.a.: 143-162)
hKGF-10:  THNGGEMFVALNQKGIPVRG (a.a.: 157-176)
hKGF-11:  GIPVRGKKTKKEQKTAHFLPMAIT (a.a.: 171-194)
```

| Peptide | Purity (%) | Quantity (mg) |
|---|---|---|
| hKGF-1 | 98.65 | 10 |
| hKGF-2 | 97.56 | 15 |
| hKGF-3 | 88.26 | 3 |
| hKGF-4 | 89.62 | 10 |
| hKGF-5 | 96.79 | 10 |
| hKGF-6 | 63.84 | 2 |
| hKGF-7 | 99.94 | 10 |
| hKGF-8 | 96.43 | 20 |
| hKGF-9 | 93.57 | 15 |
| hKGF-10 | 99.30 | 15 |
| hKGF-11 | 97.05 | 10 |

*Task 1.2: establishment of in vitro model systems for initial screening (Months 3-15)*

*Task 1.2.1: development of stably-transfected MCF-7 cells with KGF (Months 1-3)*

A stably-transfected MCF-7 cell line with KGF was developed. After propagation, this cell line is cryopreserved and stored in a liquid nitrogen container for further use.

*Task 1.2.2: development of stably-transfected MDA-MB-231 cells with KGF (Months 2-4)*

A stably-transfected MDA-MB-231 cell line with KGF was developed. After propagation, this cell line is cryopreserved and stored in a liquid nitrogen container for further use.

*Task 1.3: Evaluation of synthsized KGF antagonists in stably-transfected MCF-7 (Months 3-9)*

*Task 1.3.1: $^{3}$H-thymidine incorporation assay (Months 3-7)*

The optimum condition for DNA synthesis assay was established, however due to lack of synthetic peptide quantity for this kind of assay, $^{3}$H-thymidine incorporation assay was not carried out. This assay will be performed after receptor binding assay will have been carried out.

*Task 1.3.2: aromatase activity assay (Months 3-7)*

The optimum condition for DNA synthesis assay was established, however due to lack of synthetic peptide quantity for this kind of assay, aromatase activity assay was not carried out. This assay will be performed after receptor binding assay will have been carried out.

*Task 1.3.3: receptor binding assay (Months 4-9)*

Since this assay needs smallest quantity of synthesized peptide antagonist, we have decided to perform the receptor binding assay first. The MCF-7 cells were used for this binding assay. Iodinated KGF was synthesized and used for this assay. However, the receptor to KGF (KGFR) expression was not high enough to obtain the consistent receptor binding assay results. Therefore, we have modified to increase the sensitivity of assay. The outside membrane portion of the KGFR was PCR cloned into the pEF6/V5-His-TOPO high-level expression vector. After DNA sequence was confirmed, the MCF-10a human normal breast epithelial was transfected with this plasmid and stably transfected cells were selected and isolated for further use. Since this overexpressed protein have a V5-tag immediately after the cloned outside membrane portion of KGFR, a 96-well plate well could be coated with this over expressed protein along with V5 antibody which we have. A well, which is coated with the known amount of overexpressed protein, will be used for a receptor binding assay (competition assay). This modification will significantly increase the sensitivity of this assay.

Currently, we are in the process to optimize the assay condition.

*Task 1.4: Evaluation of synthsized KGF antagonists in stably-transfected MDA-MB-231 (Months 5-11)*

See above section for detail.

*Task 1.4.1: $^{3}$H-thymidine incorporation assay (Months 5-9)*

See above section for detail.

*Task 1.4.2: aromatase activity assay (Months 5-9)*

See above section for detail.

*Task 1.4.3: receptor binding assay (Months 6-11)*

See above section for detail.

*Task 1.5: Evaluation of synthsized KGF antagonists in primary cultured human breast epithelial cells (Months 7-20)*

See above section for detail.

*Task 1.5.1: $^{3}$H-thymidine incorporation assay (Months 7-20)*

See above section for detail.

*Task 1.5.2: aromatase activity assay (Months 7-20)*

See above section for detail.

*Task 1.5.3: receptor binding assay (Months 7-20)*

See above section for detail.

***Task 2: KGF peptide Three-D structural analysis (Months 1-3)***

*Task 3: Syntheses of a series of small peptides based on 3-D structural analysis results (Months 4-5)*

Due to the difficulties of peptide syntheses described above, this section is not initiated.

*Task 3.1: Analyses of synthetic small peptides will be performed (Month 4)*

*Task 3.2: Evaluation of synthsized KGF antagonists in stably-transfected MCF-7 (Months 3-9)*

*Task 3.2.1: $^{3}$H-thymidine incorporation assay (Months 3-7)*

*Task 3.2.2: aromatase activity assay (Months 3-7)*

*Task 3.2.3: receptor binding assay (Months 4-9)*

*Task 3.3: Evaluation of synthsized KGF antagonists in stably-transfected MDA-MB-231 (Months 5-11)*

*Task 3.3.1: $^{3}$H-thymidine incorporation assay (Months 5-9)*

*Task 3.3.2: aromatase activity assay (Months 5-9)*

*Task 3.3.3: receptor binding assay (Months 6-11)*

*Task 3.4: Evaluation of synthsized KGF antagonists in primary cultured human breast epithelial cells (Months 9-22)*

*Task 3.5.1: $^{3}$H-thymidine incorporation assay (Months 9-22)*

*Task 3.5.2: aromatase activity assay (Months 9-22)*

*Task 3.5.3: receptor binding assay (Months 9-22)*

*Task 4: Syntheses of a series of modified small peptides (Months 18-20)*

*Task 4.1: Analyses of synthetic modified small peptides will be performed (Month 20)*

*Task 4.2: Evaluation of synthsized KGF antagonists in stably-transfected MCF-7 (Months 20-32)*

*Task 4.2.1: $^{3}$H-thymidine incorporation assay (Months 20-32)*

*Task 4.2.2: aromatase activity assay (Months 20-32)*

*Task 4.2.3: receptor binding assay (Months 20-34)*

*Task 4.3: Evaluation of synthsized KGF antagonists in stably-transfected MDA-MB-231 (Months 20-32)*

*Task 4.3.1: $^{3}$H-thymidine incorporation assay (Months 20-32)*

*Task 4.3.2: aromatase activity assay (Months 20-32)*

*Task 4.3.3: receptor binding assay (Months 20-32)*

*Task 4.4: Evaluation of synthsized KGF antagonists in primary cultured human breast epithelial cells (Months 24-36*

*Task 4.4.1: $^{3}$H-thymidine incorporation assay (Months 24-36)*

*Task 4.4.2: aromatase activity assay (Months 24-36)*

*Task 4.5.3: receptor binding assay (Months 24-36)*

## KEY RESEARCH ACCOMPLISHMENTS

- Eleven potential synthetic peptide-antagonists were developed
- Stably transfected human breast cancer cell lines were established
- Primary cultured human non-cancerous breast epithelial cells were established
- Primary cultured human cancerous breast epithelial cells were established

## REPORTABLE OUTCAMS

- Abstract

  **FGF-10 stimulates DNA synthesis and aromatase activity in primary cultured breast epithelial cells and MCF-7 cells**, Yasuro Sugimoto, Samuel K. Kulp, Kyong Lee, Robert W. Brueggemeier, and Young C. Lin, American Association for Cancer Research 2000.

  Please see next page for a copy of the abstract.

- development of cell lines

  Stably transfected MCF-7 cells with the plasmid pcDNA3.1(-)-hKGF.
  Stably transfected MDA-MB-231 cells with the plasmid pcDNA3.1(-)-hKGF.
  Primary cultured human breast epithelial cells.

## CONCLUSIONS

Eleven potential peptide-antagonists were synthesized. Due to lack of sensitivity, originally proposed assay systems were not used for their evaluation, however, modified evaluation system is on the process of development. The cell lines needed for further studied were established and primary cultured human breast epithelial cells are isolated and cryopreserved for future study.

The FGF-10, which shares the same receptor, KGFR, was reported. FGF-10 shares high homology with KGF amino acid sequences. Therefore, FGF-10 sequence will be included for computer 3-D structural analysis.

# American Association for Cancer Research 2000
# Abstract Proof Page

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**FGF-10 stimulates DNA Synthesis and Aromatase Activity in primary cultured breast epithelial cells and MCF-7 cells**

Yasuro Sugimoto, Samuel K Kulp, Kyong Lee, Robert W Brueggemeier, Young C Lin, OSU Comprehensive Cancer Ctr and Ohio State Univ, Columbus, OH; Ohio State Univ, Columbus, OH

Estrogens promote growth of estrogen responsive breast cancer cells. Production of growth factors by stimulation of estrogens in breast cancer cells has been shown to date. Our recent findings suggest that KGF/FGF-7 is capable of acting as a mediator of the stimulatory effects of estrogen in human breast cancer cells. FGF-10 is a newly identified member of the FGF family and is a homologue of KGF. Thus, we hypothesize that in breast cancer cells, FGF-10 is able to stimulate cell growth and induce aromatase(Arom) activity. MCF-7 cells and primary cultured normal human breast epithelial cells (EC) were treated with recombinant FGF-10 for 24 hours at increasing dose levels. DNA synthesis was measured by using [$^3$H] thymidine incorporation assay and BrdU-incorporation followed by ELISA. Arom activity was determined by measuring the amount of $^3H_2O$ released into culture medium after cells were pulsed with [1$\beta$-$^3$H] androstenedione. DNA synthesis was significantly elevated by 79.52% at a FGF-10 conc of 10ng/ml in MCF-7 cells. Arom activity was also significantly increased at 10ng/ml by 79.25% in MCF-7 cells. In EC, FGF-10 stimulated DNA synthesis by 53.21% after 24hours treatment. Arom activity was also measured after 72hours treatment and was elevated by 68.26%, however, elevation of Arom activity after 24hours treatment was negligible. Our results suggest that similarity between FGF-10 and KGF is not only structural but also functional in cultured breast cells. Since FGF-10, like KGF, is reported to be expressed among stromal cells in tissues and to bind to its receptor in epithelial cells, both growth factors may play a significant role in breast cell function and proliferation (Supported: USArmyDOD DAMD179919341and DAMD8140, and NIH CA66193)